REMARKS

Reconsideration of the rejections set forth in the Office action mailed May 11, 2004 is respectfully requested. Claims 1-18 are currently under examination; claims 19-34 have been canceled.

I. Claim to Foreign Priority

Applicants acknowledge the requirement to file a certified copy of the priority document. In accordance with 37 CFR §1.55(a)(2) and MPEP §201.13(II)(A), a certified copy of the priority document will be filed before the grant of the patent.

II. Amendments

The specification has been amended to add section headings and to include a Brief Description of the Drawings, as requested by the Examiner, following the Summary of the Invention. The text of the description of the drawings is taken from the more detailed description of the drawings on pages 42-45 of the specification as filed.

Claims 1, 3-8, 10 and 16 have been amended for clarity. These amendments are described further, in response to each of the Examiner's objections, in Sections IV and V below.

Claims 1 and 4 are also amended to recite that the nucleic acid carries a means for immobilizing the nucleic acid to a solid support at the 5' end; that the colony primer X carries a means for immobilizing the colony primer to a solid support at the 5' end; and that in step (2) the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support. Dependent claims 10 and 11 are accordingly amended to replace the term "attaching" with "immobilizing". Support is found, for example, at page 14, lines 19-20; page 20, lines 8-9 and 23-24; page 22, lines 5-6; and page 23, lines 23-24 and 35.

No new matter is added by any of the amendments.

III. Amendments to the Drawings

Enclosed is one sheet of replacement drawings, labeled "Replacement Sheet", to replace

original sheet 2/10 ("Fig. 1 cont'd"), plus one sheet of marked-up drawings, labeled "Annotated Marked-up Drawings", showing the changes made in red.

The amendments correct obvious errors in the drawing. The amendments are consistent with the description of the Figure at page 25, line 33 to page 26, line 15 of the specification (emphasis added):

> When primer extension is complete, see Figure 1(e), it can be seen that a second immobilised nucleic acid strand has been generated which is complementary to the initial nucleic acid template. On separating the two nucleic acid strands...two immobilised nucleic acids will be present, *the first being the initial immobilised nucleic acid template and the second being a nucleic acid complementary thereto,* extending from one of the immobilised colony primers X, see *Figure 1(f)*.
>
> *Both the original immobilised nucleic acid template and the immobilised extended colony primer formed are then able to hybridise to other colony primers present (depicted as colony primers 2 and 3 in Figure 1(g))* and after a further round of primer extension (Figure 1(h)) and strand separation (*Figure 1(i)*), four single stranded immobilised strands are provided. *Two of these contain sequences corresponding to the original nucleic acid template and two contain sequences complementary thereto.*

In view of the above description, the nucleic acid strand depicted on the right side of Figure 1(f) has been corrected to correspond with the same strand shown (in annealed form) in the preceding Figure 1(e), shown on drawing sheet 1/10. This second strand is complementary to the strand on its left (the "initial immobilised nucleic acid template"), as described above (*i.e.* "the second being a nucleic acid complementary thereto").

Figure 1(g) has been corrected to correspond with the description stating that "Both the original immobilised nucleic acid template and the immobilised extended colony primer formed are then able to hybridise to other colony primers present (depicted as colony primers 2 and 3 in Figure 1(g))". That is, Figure 1(g) is corrected so that the primer, rather than the nucleic acid template, is labeled "2" and is attached to the solid support. This also corresponds with the subsequent Figure 1(h).

The sequence of the strand on the right side of Figure 1(g) is also corrected to correspond to the same strand as shown in Figure 1(f) and in Figure 1(h).

Finally, the first and third strands in Figure 1(i) are corrected to correspond to same strands shown (in annealed form) in the preceding Figure 1(h). This also corresponds with the description above, which states that, in Figure 1(i), "Two of these [strands] contain sequences corresponding to the original nucleic acid template and two contain sequences complementary thereto."

As stated above, the amendments and the amended drawing are consistent with the description in the specification as filed. Therefore, no new matter is added by the amendments.

IV. Objection to the Specification

The specification has been amended to include a Brief Description of the Drawings, as requested by the Examiner, following the Summary of the Invention. The text of the brief description of the drawings is taken from the more detailed description of the drawings on pages 42-45 of the specification as filed.

V. Claim Objections

Each of the items in the Office Action is addressed as follows.

Item 4. Claim 1 has been amended as suggested by the Examiner.

Item 5. It seems to the applicants to be unambiguous that "step (2)" in dependent claim 3 must refer to step (2) of parent claim 1. However, because the meaning and scope of the claim is not affected by the amendment, the claim has been amended as suggested by the Examiner.

Item 6. Claim 4 has been amended as suggested by the Examiner.

Item 7. It is unclear to the applicants why the phrase "colonies generated" in claims 5, 8, and 16 is objected to, since the parent claim recites that "nucleic acid colonies are generated". However, because the meaning and scope of the claims is not affected by the amendment, the claims have been amended as suggested by the Examiner.

VI. Rejections under 35 U.S.C. §112, Second Paragraph

Claims 3 and 5-13 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Each of the items in the Office Action is addressed individually below.

Item 10. Claim 3 has been amended to recite that "the sequences of the two different colony primers X are such that the oligonucleotide sequence Z can hybridise to one of the colony primers X and the oligonucleotide sequence Y is the same as the sequence of one of the colony primers X". It is thus clear that "the sequences" refers to the sequences of the two different primers and not to two sequences within one primer.

Item 11. Claim 5 has been amended as suggested by the Examiner.

Item 12. For clarification, claim 5 has been further amended to recite "sequence determination of nucleic acid templates in one or more of the nucleic acid colonies" instead of "sequence determination of one or more of the nucleic acid colonies". Support is found, for example, at page 30, lines 1-11, particularly 11, and page 30, line 35 of the specification.

Item 13. Claim 6 has been amended to change "the incorporation" to "incorporation", since this is a new element introduced in the claim.

Item 14. The term "labelled nucleotide" (which has been corrected from the original "labelled oligonucleotides", in accordance with, for example, page 30, lines 18-20 of the specification) is a new element introduced in dependent claim 6. Sequencing of nucleic acids by incorporation and detection of labeled nucleotides is known in the art and is described in the specification on pages 30-32 and in Example 5.

Item 15. The term "the amplified nucleic acid templates" in claim 7 has been amended to "nucleic acid templates", which term is used in parent claim 5, as amended.

Item 16. The Examiner stated that it was unclear whether "said solid support" in claim 10 means "a solid support for attaching the nucleic acid or a solid support for the colony primers".

Claim 10, as previously presented, recited "wherein the means for attaching the nucleic acid template and the colony primers to *the solid support* comprises a means for attaching

the nucleic acid sequences covalently to *the said support*". As currently amended, the claim recites "wherein the means for immobilizing the nucleic acid template and the colony primers to *the solid support* comprises a means for immobilizing the nucleic acid template and the colony primers covalently to *the said support*".

In both versions of the claim, the language "to *the* solid support" and "to *the said* support" makes it clear that both species (that is, "the nucleic acid template and the colony primers") are attached/immobilized to the same support.

Item 17. The applicants do not agree that "claim 1 does not require a support". Step (2) of the claim, as amended, recites that "the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support"; as previously presented, it recited that that "the 5' ends of both the nucleic acid template and the colony primers bind to the solid support". The references to the support in claims 14-18 are thus entirely clear.

In view of the foregoing, the applicants submit that the pending claims comply with the requirements of 35 U.S.C. §112, second paragraph, and request that the rejections under this section be withdrawn.

VII. Rejections under 35 U.S.C. §102(b)

Claims 1-3, 10-12, 14 and 15 were rejected under 35 U.S.C. §102(b) as being anticipated by Adams *et al.*, WO 96/04404. This rejection is respectfully traversed for the following reasons.

A. The Invention

The applicant's invention, as embodied in claim 1, is directed to a method for amplification of at least one nucleic acid, comprising the following steps:

(1) forming at least one nucleic acid template comprising the nucleic acid to be amplified, wherein the nucleic acid contains an oligonucleotide sequence Y at the 5' end and an oligonucleotide sequence Z at the 3' end, and the nucleic acid carries a means for immobilizing the nucleic acid to a solid support at the 5' end;

(2) mixing the at least one nucleic acid template, in the presence of a solid support, with one or more colony primers X, each of which can hybridize to the oligonucleotide sequence

Z and carries a means for immobilizing the colony primer to a solid support at the 5' end, whereby the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support; and

(3) performing one or more nucleic acid amplification reactions on the immobilized nucleic acid template, so that nucleic acid colonies are generated.

With reference to immobilization of the nucleic acid template and colony primers to the solid support (step (2) of the claim), the applicants' specification states as follows (page 14, lines 19-27) (emphasis added):

> "Attachment" relates to *immobilization* of nucleic acid on solid supports by either a covalent attachment or via irreversible passive adsorption or via affinity between molecules (for example, immobilization on an avidin-coated surface by biotinylated molecules). The attachment must be of sufficient strength that *it cannot be removed by washing with water or aqueous buffer under DNA-denaturing conditions*.

B. The Prior Art

The cited reference, Adams *et al.*, does not teach step (2) of applicants' claimed method, in which colony primers are mixed with nucleic acid templates in the presence of a solid support, "whereby the 5' ends of both the nucleic acid template and the colony primers are *immobilized* to the solid support".

In particular, Adams does not show a method in which nucleic acid templates are "*immobilized* to the solid support" (step (2) of applicants' claim) prior to the step of "performing one or more nucleic acid amplification reactions" on the nucleic acid templates (step (3) of applicants' claim).

As shown in Figures 1 and 2 of Adams, for example, it is clear that, prior to any amplification reaction, nucleic acid templates are bound only by hybridization to the primer.

For example, with respect to Figure 1D, the Adams reference states that "FIG. 1D depicts *an annealed, or hybridization product* comprising first nucleic acid strand **25**" (page 15, lines 9-11; emphasis added). Subsequently, "Upon imposition of denaturation

conditions, *a denaturation product is formed* comprising first nucleic acid strands **25** and **27**" (page 16, lines 6-8; Fig. 1F; emphasis added). Fig. 1F clearly shows that strand **25** is no longer attached to the solid support.

(Note that in Adams, the nucleic acid template, *i.e.* the target sequence to be amplified, is referred to as the "first nucleic acid", and the immobilized primers are referred to as "second" and/or "third" nucleic acids. See *e.g.* page 14, lines 5-15 and 29-30.)

With respect to Fig. 2C, the Adams reference states that "an *annealed product* is formed in area **121** comprising a first nucleic acid **133** and a second nucleic acid **125**" (page 19, lines 6-8; emphasis added). Subsequently, "the fifth work station imposes *denaturation conditions* on the elongation product **145** *to allow first nucleic acid strand **133** to dissociate* from second nucleic acid **125**" (page 19, lines 33-35; emphasis added).

Because the nucleic acid template in Adams "dissociates" from the primer under denaturation conditions, it cannot be considered "immobilized". Thus, Adams *et al.* does not teach immobilization of the template nucleic acid to the solid support prior to amplification.

Since the reference does not disclose all of the elements set out above in independent claim 1, this claims and its dependent claims cannot be anticipated by this reference under 35 U.S.C. §102(b). In view of this, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §102(b).

VIII. Rejections under 35 U.S.C. §102(e)

Claims 1-3, 10-12, 14, 15 and 17 were rejected under 35 U.S.C. §102(e) as being anticipated by Adams *et al.*, U.S. Patent No. 6,060,288. This rejection is respectfully traversed for the following reasons.

The disclosure of this U.S. patent is very similar to that of the Adams *et al.* PCT publication discussed above, with which it shares priority, with the exception of new Examples 5-8 in the U.S. patent. Applicants note that the Examiner's characterization of the U.S. patent (pages 9-13 of Office Action) is essentially identical to the Examiner's characterization of the PCT publication (pages 5-9 of Office Action), with the exception of

cited column and line numbers, and the reference to applicants' dependent claim 17.

With respect to the invention of independent claim 1, there is no substantial difference between the disclosures of these two references. Therefore, for the same reasons discussed above, Adams *et al.* does not teach step (2) of applicants' claimed method, in which colony primers are mixed with nucleic acid templates in the presence of a solid support, "whereby the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support".

In particular, Adams clearly does not show a method in which nucleic acid templates are "immobilized to the solid support" (step (2) of applicants' claim) prior to the step of "performing one or more nucleic acid amplification reactions" on the nucleic acid templates (step (3) of applicants' claim).

Since the reference does not disclose all of the elements set out above in independent claim 1 and its dependent claims, these claims cannot be anticipated by this reference under 35 U.S.C. §102(e). In view of this, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §102(e).

IX. Rejections under 35 U.S.C. §103(a)

Claim 4 was rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Huang, U.S. Patent No. 5,645,994.

Claims 5, 6, 8, and 9 were rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Bukh *et al.*, U.S. Patent No. 5,514,539.

Claim 7 was rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Bukh *et al.* (above) and further in view of Hildebrand *et al.*, U.S. Patent No. 6,287,764.

Claim 13 was rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Lund *et al.*, *Nucleic Acids Research* **16**:10861-80 (1988).

Claims 16 and 18 were rejected under 35 U.S.C. §103(a) as being unpatentable over Adams *et al.*, 1996 (above) in view of Fodor *et al.*, U.S. Patent No. 5,800,992.

The rejections are respectfully traversed in light of the following remarks.

A. The Invention

The invention of independent claims 1 and 4, as described above, includes the steps of:

"(2) mixing...at least one nucleic acid template, in the presence of a solid support, with one or more [degenerate] colony primers...whereby the 5' ends of both the nucleic acid template and the colony primers are immobilized to the solid support; and

(3) performing one or more nucleic acid amplification reactions on the immobilized nucleic acid template, so that nucleic acid colonies are generated".

Accordingly, the invention is directed to an amplification method in which, prior to amplification, the nucleic acid template is immobilized to a solid support. As discussed above, in Section VI of this response, such a method is not taught in the primary cited reference, Adams *et al.*

The applicants' specification describes the different effects that can be achieved via this "initial immobilization" of the template, in contrast to prior art methods in which the template is not immobilized:

> The initial immobilisation of the template nucleic acid means that the template nucleic acid can only hybridise with colony primers located at a distance within the total length of the template nucleic acid. Thus the boundary of the nucleic acid colony formed is limited to a relatively local area to the area in which the initial template nucleic acid was immobilised. (page 23, line 35 to page 24, line 4)
>
> It can thus be seen that the method of the present invention allows the generation of a nucleic acid colony from a single immobilised nucleic acid template and that the size of these colonies can be controlled by altering the number of rounds of amplification that the nucleic acid template is subjected to. Thus the number of nucleic acid colonies formed on the surface of the solid support is dependent upon the number of nucleic acid templates which are initially immobilised to the support....
>
> Such so called "autopatterning" of nucleic acid colonies has an advantage over many methods of the prior art in that a higher density of nucleic acid colonies can be obtained due to the fact that the density can be controlled by regulating the

density at which the nucleic acid templates are originally immobilised. Such a method is thus not limited by, for example, having specifically to array specific primers on particular local areas of the support and then initiate colony formation by spotting a particular sample containing nucleic acid template on the same local area of primer. The numbers of colonies that can be arrayed using prior art methods, for example those disclosed in W096/04404 (Mosaic Technologies, Inc.) is thus limited by the density/spacing at which the specific primer areas can be arrayed in the initial step (page 26, line 21 to page 27, line 17).

B. The Cited Art

As established in Section VII of these Remarks, neither the primary cited reference, Adams *et al.*, 1996 (WO 96/04404), nor its U.S. equivalent, U.S. Patent No. 6,060,288, teaches the method of independent claim 1.

Nor does Adams *et al.* provide any motivation to alter the disclosed method along the lines of the applicants' invention, by immobilizing the nucleic acid template to the solid support. The method of Adams *et al.* is described as useful for "the detection of the presence of (or the absence of) a target nucleic acid sequence in a test sample" (column 1, lines 50-54 of US patent). In preferred embodiments, the method "further comprises the step of monitoring the support for the presence of one or more amplification products, in which one or more amplification products are indicative of the presence of one or more target sequences, and in which *absence* of an amplification product is indicative of the *absence* of a target sequence" (page 6, lines 30-34 of PCT; claim 2 of PCT; sentence bridging columns 3-4 of US patent; emphasis added).

Because the method of Adams *et al.* is intended to indicate when a target sequence is absent from a sample, there would clearly be no reason to immobilize the target sequence to the support, since this would produce a false positive result, indicating that the target sequence was present.

The secondary references, discussed briefly below, were cited for their disclosure of various individual features not recited in independent claim 1. However, none of these references make up for the deficiencies of the primary reference(s) with respect to the

applicants' independent claims. That is, none of them discloses or suggests an amplification method in which a nucleic acid template and primers are immobilized to a solid support prior to amplification. Therefore, the combination of any or all of these references with Adams *et al.* does not disclose or suggest the claimed amplification method.

Huang, which is concerned with identification of disease-causing microbes in biological samples (as stated in the Field of the Invention), is cited for the disclosure of "universal primers". However, the "universal primers" in Huang are not in fact fully degenerate primers, since they are designed based on a protein which is ubiquitous in species to be detected in a sample (*e.g.* bacterial or microbial species) and which has both highly conserved and variable sequences. Preferably, the protein is type II topoisomerase, and the primers are based on highly conserved regions of this protein (column 3, line 66 to column 4, line 29).

Bukh *et al.*, which is directed to identification and uses of envelope 1 gene sequences of HCV isolates, is cited for the disclosure of sequencing of PCR products and the use of labeled primers for detection of the amplification products. A conventional method of sequencing (dideoxy-nucleotide chain termination method) is used in Example 1 of Bukh *et al.* However, sequencing by to incorporation and detection of labeled nucleotides (as recited in applicants' claim 6) is not described in Bukh *et al.*

Adams *et al.*, the primary reference, is further cited for disclosure of the visualization of nucleic acid colonies, which may employ a labeled or unlabeled nucleic acid probe.

Hildebrand *et al.*, which is directed to methods of HLA Class I typing by sequencing, is cited for its disclosure of simultaneous sequencing of PCR products. In the passage pointed out by the Examiner, ten PCR products are sequenced simultaneously using the "AutoLoad Solid Phase Sequencing Kit". Because the PCR products must be bound to "combs" provided with the kit (column 12, lines 37-43), this procedure would be less than ideal for sequencing PCR products which are already bound to a solid support.

Lund *et al.*, which is directed to a study of methods of attaching nucleic acids to magnetic beads, is cited for its disclosure of the use of a 5'-amino group for attachment of a nucleic acid to carboxyl-functionalized magnetic beads.

Fodor *et al.*, which is directed to the use of large arrays of nucleic acids for sequencing, is cited for its disclosure of supports having attached nucleic acids at a density of 10,000-100,000/mm^2. Such supports are prepared by use of "masking technology and photosensitive synthetic subunits (column 7, lines 55-56).

The teachings of these references, directed to various technologies, provide no guidance regarding a method in which a nucleic acid template and primers are immobilized to a solid support, followed by amplification of the template. No combination of the teachings of the secondary references with those of Adams *et al.* would have suggested the claimed method to one skilled in the art.

In view of the foregoing, the applicants respectfully request the Examiner to withdraw the rejections under 35 U.S.C. §103(a).

X. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,

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